09/554784

PATENT COOPERATION TREATY

REC'D 21 MARS 2000

WIPO PCT

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
BO 41356		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/NL98/00663	19/11/1998	19/11/1997
International Patent Classification (IPC) or national classification and IPC C07K16/00		
Applicant TANOX PHARMA B.V. et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 9 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☒ Certain documents cited
   - VII ☒ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
17/06/1999	16.03.00
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Maucher, C Telephone No. +49 89 2399 7415

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/NL98/00663

I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1-13 as originally filed

Claims, No.:

1-10 as originally filed

Drawings, sheets:

1/3-3/3 as originally filed

2. The amendments have resulted in the cancellation of:

☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 10 with respect to industrial applicability.

because:

☒ the said international application, or the said claims Nos. 10 relate to the following subject matter which does not require an international preliminary examination (*specify*):

see separate sheet

☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for the said claims Nos. .

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims 5-7, 9
	No:	Claims 1-4, 8, 10
Inventive step (IS)	Yes:	Claims 5
	No:	Claims 1-4, 6-10
Industrial applicability (IA)	Yes:	Claims 1-9
	No:	Claims

2. Citations and explanations

see separate sheet

VI. Certain documents cited

1. Certain published documents (Rule 70.10)

and / or

2. Non-written disclosures (Rule 70.9)

see separat sh t

VII. Certain defects in th international application

The following defects in the form or contents of the international application have been noted:

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Point III:

Claim 10 relates to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of this claim (Article 34(4)(a)(I) PCT).

Point V:

The following documents (D) are referred to in this communication; the numbering will be adhered to in the rest of the procedure:

D1: EP-A-0 759 466

D1 discloses the production of monoclonal antibodies against the human IL-12 beta2 receptor protein or fragment thereof by known methods. These antibodies can be used to prevent or treat pathological conditions caused by excess activity of cells possessing IL-12 receptors by inhibiting binding of IL-12 to such cells (page 7, line 56-59). These antibodies can be used in combination with other cytokine antagonists such as antibodies to the IL-2 receptor etc. as a pharmaceutical composition (page 8, line 13-19; claim 24). The antibody can be used for the preparation of a medicament ... for the treatment of autoimmune dysfunction (page 8, line 20-21).

1. Novelty

1.1. The subject-matter of claims 1-3, 8 and 10 does not seem to be novel (Article 33(2) PCT) in view of D1. The only distinguishing feature is the result to be achieved, i.e. the prevention of IL12R β2 chain-mediated STAT4 phosphorylation and/or the prevention of dimerization of the IL12R β2 chain to the IL12R β1 chain. It is possible that the monoclonal antibodies disclosed in D1 have the same activities (mentioned above) as the ones claimed in the present application, which would render the claimed subject-matter known in the art.
This objection cannot be solved by restricting the claims to a specific monoclonal

antibody, since the only mentioned monoclonal antibody 3H4 (mentioned in Example 4, pages 11-12) is not sufficiently disclosed (neither a reference to an internationally recognized deposit, nor the sequence of said antibody could be found in the application) (see Article 5 and Rule 13bis PCT). No monoclonal antibody binding to the IL12R β2 chain is shown in comparison which is not able to prevent the said STAT phosphorylation. Thus, the claimed antibody is not shown to be exceptional among the different monoclonal antibodies binding to the IL12R β2 chain due to its ability to prevent the said STAT phosphorylation.

1.2. Claim 4 is not considered to be novel (Article 33(2) PCT), since D1 discloses a complex of monoclonal antibodies and human IL12R β2 (page 7, lines 56-57). The IPEA believes that, besides every antigen capable of being an autoantigen, IL12R β2 receptor protein is included in the very broad term "an autoantigen ... or a modified form thereof", as disclosed in claim 4.

1.3. The subject-matter of claim 5 is considered to be novel (Article 33(2) PCT), since none of the disclosed autoantigens is mentioned in D1 (see also Point VIII 3.).

1.4. Claims 6 and 7 are considered to be novel (Article 33(2) PCT) in view of D1, since their subject-matter is distinguished from D1 in that a second "monoclonal" antibody is used.

1.5. The subject-matter of claim 9 is considered to be novel according to Article 33(2) PCT, since it is distinguished from D1 in that "a heat shock protein or peptide fragments of said heat shock protein" for the stimulation of type 2 cytokine producing regulatory T cells is claimed (see also Point VIII 3.).

2. Inventive step

2.1. Although the technical problem to be solved by claim 5 is not explicitly stated in the application, it seems to be how to provide a combination for a simultaneous treatment of the symptoms and the disease process in specific autoimmune diseases.

The problem is solved by combining the antibody of any one of claims 1-3 with specific autoantigens.

This solution could not be derived from any prior art document, either if taken alone or in any combination. Thus, the subject-matter of claim 5 is considered to meet the requirements of Article 33(3) PCT except for the feature "heat shock proteins" (see also Point VIII 3.).

2.2. The subject-matter of claim 6 is not considered to be inventive (Article 33(3) PCT) in view of the combination of 2 antibodies disclosed in D1 (see summary above). The present application does not describe any surprising effect provoked by the second antibody being explicitly monoclonal.

2.3. It is not apparent from the description which technical problem is solved with the subject-matter disclosed in claims 7 and 9. Furthermore, in view of D1, no surprising effect could be found in the present application upon using the combination of claim 7 or the pharmaceutical composition of claim 9. Thus, the subject-matter of claims 7 and 9 do not meet the requirements of Article 33(3) PCT (see also Point VIII, 3.).

3. Industrial applicability

For the assessment of the present claim 10 on the question whether it is industrially applicable, no unified criteria exist in the PCT contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

Point VI:

1. The subject-matter of the interfering document "WO-A-9841232" refers to relevant

subject-matter.

The above document is published after the present application's priority date, but before its filing date and is therefore relevant for those parts of the present application, if any, which do not have a valid claim to priority.

2. Certain published documents (Rule 70.10)

Application No Patent No	Publication date *(day/month/year)*	Filing date *(day/month/year)*	Priority date *(valid claim)* *(day/month/year)*
US-A-5852176	22.12.1998	20.8.1997	-
US-A-5853721	29.12.1998	31.1.1995	-

Should the invention appear not to be entitled to the claimed priority date, the above documents should be taken into consideration when examining whether the invention is new and involves an inventive step.

Point VII:

1. Contrary to the requirements of Rule 5.1(a)(ii) PCT, the document D1 has not been identified in the description and the relevant background art disclosed therein has not been briefly discussed.

2. The applciant should take care during revision, especially of the introductory portion and any statements of problem or advantage, not to add subject-matter which extends beyond the content of the application as originally filed (Articles 19(2) and 34(2)(b) PCT).

3. In order to facilitate the examination of the conformity of the amended application with the requirements of Articles 19(2) and 34(2)(b) PCT, the applicant is requested to clearly identify the amendments carried out, irrespective of whether they concern amendments by addition, replacement or deletion, and to indicate the passages of the application as filed on which these amendments are based.

These indications should be submitted in handwritten form on a copy of the relevant parts of the application as filed.

Point VIII:

1. The term "or a modified form thereof" used in claim 4 is vague and unclear and leaves the reader in doubt as to the meaning of the technical features to which it refers, thereby rendering the definition of the subject-matter of said claim unclear (Article 6 PCT). It is furthermore unclear whether this term relates to an autoantigen or peptide fragments of an autoantigen.

2. The term "any one of claims 1" in claim 6 is unclear (Article 6 PCT) and should be corrected.

3. Claims 5 and 9 are not clear (Article 6 PCT), since the subject-matter "heat shock protein (or peptide fragments of said heat shock protein)" is not supported by the description.

4. Claims 6 and 7 are not clear (Article 6 PCT), since it is not apparent from the description, e.g. from an experiment, to which effect the combined use of the subject-matter of any one of claims 1-3 with the monoclonal antibodies is leading.

5. Claim 9, which is defined by the result to be achieved, and claim 8 are unclear (Article 6 PCT), since the description does not show any effect of the simultaneous use of an antibody from claim 1, 2 or 3 and the heat shock protein or peptide fragment, or the combination of claim 8. No experiment is disclosed proving the effect (Article 5 PCT).

PATENT COOPERATION TREATY 09/554784

PCT

NOTIFICATION OF RECEIPT OF RECORD COPY

(PCT Rule 24.2(a))

From the INTERNATIONAL BUREAU

To:

DE BRUIJN, Leendert, C.
Nederlandsch Octrooibureau
Scheveningseweg 82
P.O. Box 29720
NL-2502 LS The Hague
PAYS-BAS

Date of mailing (day/month/year)	IMPORTANT NOTIFICATION
10 December 1998 (10.12.98)	
Applicant's or agent's file reference	**International application No.**
BO 41356	PCT/NL98/00663

The applicant is hereby notified that the International Bureau has received the record copy of the international application as detailed below.

Name(s) of the applicant(s) and State(s) for which they are applicants:

TANOX PHARMA B.V. (for all designated States except US)
DE BOER, Mark et al (for US)

International filing date	:	19 November 1998 (19.11.98)
Priority date(s) claimed	:	19 November 1997 (19.11.97)
Date of receipt of the record copy by the International Bureau	:	08 December 1998 (08.12.98)
List of designated Offices	:	

EP :AT,BE,CH,CY,DE,DK,ES,FI,FR,GB,GR,IE,IT,LU,MC,NL,PT,SE
National :CA,JP,US

ATTENTION

The applicant should carefully check the data appearing in this Notification. In case of any discrepancy between these data and the indications in the international application, the applicant should immediately inform the International Bureau.

In addition, the applicant's attention is drawn to the information contained in the Annex, relating to:

- [X] time limits for entry into the national phase
- [X] confirmation of precautionary designations
- [X] requirements regarding priority documents

A copy of this Notification is being sent to the receiving Office and to the International Searching Authority.

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No. (41-22) 740.14.35

Authorized officer:

P. Gonzalez

Telephone No. (41-22) 338.83.38

ANNEX TO FORM PCT/IB/301

International application No.
PCT/NL98/00663

INFORMATION ON TIME LIMITS FOR ENTERING THE NATIONAL PHASE

The applicant is reminded that the "national phase" must be entered before each of the designated Offices indicated in the Notification of Receipt of Record Copy (Form PCT/IB/301) by paying national fees and furnishing translations, as prescribed by the applicable national laws.

The time limit for performing these procedural acts is **20 MONTHS** from the priority date or, for those designated States which the applicant elects in a demand for international preliminary examination or in a later election, **30 MONTHS** from the priority date, provided that the election is made before the expiration of 19 months from the priority date. Some designated (or elected) Offices have fixed time limits which expire even later than 20 or 30 months from the priority date. In other Offices an extension of time or grace period, in some cases upon payment of an additional fee, is available.

In addition to these procedural acts, the applicant may also have to comply with other special requirements applicable in certain Offices. **It is the applicant's responsibility** to ensure that the necessary steps to enter the national phase are taken in a timely fashion. Most designated Offices do not issue reminders to applicants in connection with the entry into the national phase.

For detailed information about the procedural acts to be performed to enter the national phase before each designated Office, the applicable time limits and possible extensions of time or grace periods, and any other requirements, see the relevant Chapters of Volume II of the PCT Applicant's Guide. Information about the requirements for filing a demand for international preliminary examination is set out in Chapter IX of Volume I of the PCT Applicant's Guide.

GR and ES became bound by PCT Chapter II on 7 September 1996 and 6 September 1997, respectively, and may, therefore, be elected in a demand or a later election filed on or after 7 September 1996 and 6 September 1997, respectively, regardless of the filing date of the international application. (See second paragraph above.)

Note that only an applicant who is a national or resident of a PCT Contracting State which is bound by Chapter II has the right to file a demand for international preliminary examination.

CONFIRMATION OF PRECAUTIONARY DESIGNATIONS

This notification lists only specific designations made under Rule 4.9(a) in the request. It is important to check that these designations are correct. Errors in designations can be corrected where precautionary designations have been made under Rule 4.9(b). The applicant is hereby reminded that any precautionary designations may be confirmed according to Rule 4.9(c) before the expiration of 15 months from the priority date. If it is not confirmed, it will automatically be regarded as withdrawn by the applicant. There will be no reminder and no invitation. Confirmation of a designation consists of the filing of a notice specifying the designated State concerned (with an indication of the kind of protection or treatment desired) and the payment of the designation and confirmation fees. Confirmation must reach the receiving Office within the 15-month time limit.

REQUIREMENTS REGARDING PRIORITY DOCUMENTS

For applicants who have not yet complied with the requirements regarding priority documents, the following is recalled.

Where the priority of an earlier national, regional or international application is claimed, the applicant must submit a copy of the said earlier application, certified by the authority with which it was filed ("the priority document") to the receiving Office (which will transmit it to the International Bureau) or directly to the International Bureau, before the expiration of 16 months from the priority date, provided that any such priority document may still be submitted to the International Bureau before that date of international publication of the international application, in which case that document will be considered to have been received by the International Bureau on the last day of the 16-month time limit (Rule 17.1(a)).

Where the priority document is issued by the receiving Office, the applicant may, instead of submitting the priority document, request the receiving Office to prepare and transmit the priority document to the International Bureau. Such request must be made before the expiration of the 16-month time limit and may be subjected by the receiving Office to the payment of a fee (Rule 17.1(b)).

If the priority document concerned is not submitted to the International Bureau or if the request to the receiving Office to prepare and transmit the priority document has not been made (and the corresponding fee, if any, paid) within the applicable time limit indicated under the preceding paragraphs, any designated State may disregard the priority claim, provided that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity to furnish the priority document within a time limit which is reasonable under the circumstances.

Where several priorities are claimed, the priority date to be considered for the purposes of computing the 16-month time limit is the filing date of the earliest application whose priority is claimed.

WO 99/25737
PCT/NL98/00663

PATENT COOPERATION TREATY 09/554784

PCT

From the INTERNATIONAL BUREAU

To:
DE BRUIJN, Leendert, C.
Nederlandsch Octrooibureau
Scheveningseweg 82
P.O. Box 29720
NL-2502 LS The Hague
PAYS-BAS

NOTICE INFORMING THE APPLICANT OF THE COMMUNICATION OF THE INTERNATIONAL APPLICATION TO THE DESIGNATED OFFICES

(PCT Rule 47.1(c), first sentence)

Date of mailing (day/month/year) 27 May 1999 (27.05.99)	
Applicant's or agent's file reference BO 41356	IMPORTANT NOTICE

International application No.	**International filing date** (day/month/year)	**Priority date** (day/month/year)
PCT/NL98/00663	19 November 1998 (19.11.98)	19 November 1997 (19.11.97)

Applicant
TANOX PHARMA B.V. et al

1. Notice is hereby given that the International Bureau has communicated, as provided in Article 20, the international application to the following designated Offices on the date indicated above as the date of mailing of this Notice:
   EP,JP,US

   In accordance with Rule 47.1(c), third sentence, those Offices will accept the present Notice as conclusive evidence that the communication of the international application has duly taken place on the date of mailing indicated above and no copy of the international application is required to be furnished by the applicant to the designated Office(s).

2. The following designated Offices have waived the requirement for such a communication at this time:
   CA

   The communication will be made to those Offices only upon their request. Furthermore, those Offices do not require the applicant to furnish a copy of the international application (Rule 49.1(a-bis)).

3. Enclosed with this Notice is a copy of the international application as published by the International Bureau on
   27 May 1999 (27.05.99) under No. WO 99/25737

REMINDER REGARDING CHAPTER II (Article 31(2)(a) and Rule 54.2)

If the applicant wishes to postpone entry into the national phase until 30 months (or later in some Offices) from the priority date, **a demand for international preliminary examination** must be filed with the competent International Preliminary Examining Authority before the expiration of 19 months from the priority date.

It is the applicant's sole responsibility to monitor the 19-month time limit.

Note that only an applicant who is a national or resident of a PCT Contracting State which is bound by Chapter II has the right to file a demand for international preliminary examination.

REMINDER REGARDING ENTRY INTO THE NATIONAL PHASE (Article 22 or 39(1))

If the applicant wishes to proceed with the international application in the **national phase**, he must, within 20 months or 30 months, or later in some Offices, perform the acts referred to therein before each designated or elected Office.

For further important information on the time limits and acts to be performed for entering the national phase, see the Annex to Form PCT/IB/301 (Notification of Receipt of Record Copy) and Volume II of the PCT Applicant's Guide.

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer J. Zahra
Facsimile No. (41-22) 740.14.35	Telephone No. (41-22) 338.83.38

Form PCT/IB/308 (July 1996)

2628452

Continuati n of Form PCT/IB/308

NOTICE INFORMING THE APPLICANT OF THE COMMUNICATION OF THE INTERNATIONAL APPLICATION TO THE DESIGNATED OFFICES

Date of mailing (day/month/year) 27 May 1999 (27.05.99)	IMPORTANT NOTICE
Applicant's or agent's file reference BO 41356	International application No. PCT/NL98/00663

The applicant is hereby notified that, at the time of establishment of this Notice, the time limit under Rule 46.1 for making amendments under Article 19 has not yet expired and the International Bureau had received neither such amendments nor a declaration that the applicant does not wish to make amendments.

PATENT COOPERATION TREATY

09/554704

PCT

From the INTERNATIONAL BUREAU

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

To:

DE BRUIJN, Leendert, C.
Nederlandsch Octrooibureau
Scheveningseweg 82
P.O. Box 29720
NL-2502 LS The Hague
PAYS-BAS

Date of mailing (day/month/year)	
21 March 2000 (21.03.00)	
Applicant's or agent's file reference	**IMPORTANT NOTIFICATION**
BO 41356	
International application No.	**International filing date (day/month/year)**
PCT/NL98/00663	19 November 1998 (19.11.98)

1. The following indications appeared on record concerning:

[X] the applicant [ ] the inventor [ ] the agent [ ] the common representative

Name and Address	State of Nationality	State of Residence
TANOX PHARMA B.V. Paasheuvelweg 15 NL-1105 BE Amsterdam Netherlands	NL	NL
	Telephone No.	
	Facsimile No.	
	Teleprinter No.	

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

[ ] the person [ ] the name [X] the address [ ] the nationality [ ] the residence

Name and Address	State of Nationality	State of Residence
TANOX PHARMA B.V. Kruislaan 318 NL-1098 SM Amsterdam Netherlands	NL	NL
	Telephone No.	
	Facsimile No.	
	Teleprinter No.	

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

[X] the receiving Office
[ ] the International Searching Authority
[X] the International Preliminary Examining Authority
[ ] the designated Offices concerned
[X] the elected Offices concerned
[ ] other:

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland	Authorized officer S. Cruz
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

Form PCT/IB/306 (March 1994) 003178550

PATENT COOPERATION TREATY

09/554784

From the INTERNATIONAL BUREAU

To:

DE BRUIJN, Leendert, C.
Nederlandsch Octrooibureau
Scheveningseweg 82
P.O. Box 29720
NL-2502 LS The Hague
PAYS-BAS

INFORMATION CONCERNING ELECTED OFFICES NOTIFIED OF THEIR ELECTION

(PCT Rule 61.3)

Date of mailing (day/month/year) 16 August 1999 (16.08.99)	
Applicant's or agent's file reference BO 41356	**IMPORTANT INFORMATION**

International application No.	**International filing date** (day/month/year)	**Priority date** (day/month/year)
PCT/NL98/00663	19 November 1998 (19.11.98)	19 November 1997 (19.11.97)

Applicant
TANOX PHARMA B.V. et al

1. The applicant is hereby informed that the International Bureau has, according to Article 31(7), notified each of the following Offices of its election:

   EP :AT,BE,CH,CY,DE,DK,ES,FI,FR,GB,GR,IE,IT,LU,MC,NL,PT,SE
   National :CA,JP,US

2. The following Offices have waived the requirement for the notification of their election; the notification will be sent to them by the International Bureau only upon their request:

   None

3. The applicant is reminded that he must enter the "national phase" **before the expiration of 30 months from the priority date** before each of the Offices listed above. This must be done by paying the national fee(s) and furnishing , if prescribed, a translation of the International application (Article 39(1)(a)), as well as, where applicable, by furnishing a translation of any annexes of the international preliminary examination report (Article 36(3)(b) and Rule 74.1).

   Some offices have fixed time limits expiring later than the above-mentioned time limit. For detailed information about the applicable time limits and the acts to be performed upon entry into the national phase before a particular Office, see Volume II of the PCT Applicant's Guide.

   The entry into the European regional phase is postponed **until 31 months from the priority date** for all States designated for the purposes of obtaining a European patent.

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No. (41-22) 740.14.35

Authorized officer:
F. Baechler

Telephone No. (41-22) 338.83.38

PATENT COOPERATION TREATY

09/554784

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

DE BRUIJN, Leendert C.
NEDERLANDSCH OCTROOIBUREAU
Postbus 29720
Scheveningseweg 82
NL-2502 LS The Hague
PAYS-BAS

Nederlandsch Octrooibureau
INGEK. 21 MRT 2000
Parcaf Bewerken

preliminary examination report
opdrachtgever van client:
16-4-00
termijn omzetten in reg./nat. fase:
19-5-00

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT
(PCT Rule 71.1)

Date of mailing *(day/month/year)*	16.03.00
Applicant's or agent's file reference BO 41356	IMPORTANT NOTIFICATION

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/NL98/00663	19/11/1998	19/11/1997

Applicant
TANOX PHARMA B.V. et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/	Authorized officer
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Digiusto, M Tel.+49 89 2399-8162

Form PCT/IPEA/416 (July 1992)

PATENT COOPERATION TREATY 09/554784

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
BO 41356		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/NL 98/ 00663	19/11/1998	19/11/1997
Applicant		
TANOX PHARMA B.V. et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 4 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

[ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

[ ] contained in the international application in written form.

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[ ] furnished subsequently to this Authority in written form.

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2. [X] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

[ ] the text is approved as submitted by the applicant.

[X] the text has been established by this Authority to read as follows:

COMPOSITONS AND METHODS FOR TREATMENT OF AUTOIMMUNE DISEASES, USING A MONOCLONAL ANTIBODY TO THE INTERLEUKIN-12 BETA2-CHAIN

5. With regard to the **abstract,**

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6. The figure of the **drawings** to be published with the abstract is Figure No. _____

[ ] as suggested by the applicant.

[ ] because the applicant failed to suggest a figure.

[ ] because this figure better characterizes the invention.

[X] None of the figures.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/NL 98/00663

B x I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claim 10 is directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

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1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/NL 98/00663

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07K16/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 759 466 A (F. HOFFMAN LA ROCHE AG) 26 February 1997 cited in the application see page 8, line 9-21; claims 21-26	1-3,8,10
E	US 5 852 176 A (HOFFMAN-LA ROCHE INC.) 22 December 1998 see the whole document	1-3,8,10
E	US 5 853 721 A (HOFFMAN-LA ROCHE INC.) 29 December 1998 see the whole document	1-3,8,10
P,X	WO 98 41232 A (BASF AKTIENGESELLSCHAFT) 24 September 1998 see claims 7,13,43-57	1,8,10

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
12 April 1999	28/04/1999

Name and mailing address of the ISA	Authorized officer
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	EP 0 638 644 A (F. HOFFMAN LA ROCHE AG) 15 February 1995 cited in the application see claims 1-31	1-3,8,10
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A	PRESKY D. H. ET AL.: "A functional interleukin 12 receptor complex is composed of two beta-type cytokine receptor subunits." P.N.A.S., vol. 93, November 1996, pages 14002-7, XP002028387 see the whole document	1-3

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/NL 98/00663

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0759466 A	26-02-1997	JP 9132598 A	20-05-1997
US 5852176 A	22-12-1998	US 5840530 A	24-11-1998
US 5853721 A	29-12-1998	NONE	
WO 9841232 A	24-09-1998	AU 6760498 A	12-10-1998
EP 0638644 A	15-02-1995	US 5536657 A	16-07-1996
		AU 676325 B	06-03-1997
		AU 6750594 A	27-01-1995
		CA 2128151 A	20-01-1995
		JP 7194383 A	01-08-1995
		NZ 264003 A	21-12-1995
		ZA 9405154 A	19-01-1996
		US 5831007 A	03-11-1998
US 5639858 A	17-06-1997	AU 5368396 A	08-10-1996
		CA 2215745 A	26-09-1996
		EP 0815133 A	07-01-1998
		WO 9629341 A	26-09-1996
		US 5756700 A	26-05-1998

INTERNATIONAL SEARCH REPORT

International application No.
PCT/NL 98/00663

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

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because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

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INTERNATIONAL SEARCH REPORT

Inter. ɔnal Application No
PCT/NL 98/00663

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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INTERNATIONAL SEARCH REPORT

Inter onal Application No.
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A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07K16/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
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INTERNATIONAL SEARCH REPORT

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(74) Agent: DE BRUIJN, Leendert, C.; Nederlandsch Octrooibureau, Scheveningseweg 82, P.O. Box 29720, NL–2502 LS The Hague (NL).

(81) Designated States: CA, JP, US, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

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(54) Title: COMPOSITIONS AND METHODS FOR TREATMENT OF AUTOIMMUNE DISEASES, USING A MONOCLONAL ANTIBODY TO THE INTERLEUKIN-12 BETA2-CHAIN

(57) Abstract

Monoclonal antibodies are provided that can bind to the IL12R β2 chain expressed on the cell surface of human T lymphocytes, where said binding prevents IL12R β2 chain–mediated STAT4 phosphorylation, and/or said binding prevents the IL12R β2 chain from the formation of a complex with other membrane–associated proteins, and/or said binding prevents the IL12R β2 chain from dimerisation to the IL12R β1 chain. The antibodies may be combined with autoantigens or with antibodies to co–stimulatory receptors on T cells or antigen presenting cells. These antibodies and their combinations are also provided as pharmaceutical compositions for the treatment of autoimmune diseases.

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COMPOSITONS AND METHODS FOR TREATMENT OF AUTOIMMUNE DISEASES, USING A MONOCLONAL ANTIBODY TO THE INTERLEUKIN-12 BETA2-CHAIN

Field of the Invention

This invention relates to methods of treating diseases of in which the immune system is involved. In particular, this invention relates to methods of treating autoimmune diseases.

Background of the invention

Autoimmune diseases

One of the most intriguing characteristics of the immune system is its unlimited specificity. When threatened by potentially dangerous foreign substances (antigens), including pathogens, the immune system mounts a tailor–made response. This tailor–made response is provided by the immune systems antigen specific T and B lymphocytes. The virtually unlimited repertoire provided by these immune cell calls for a tight regulatory system preventing the recognition of our own (self) antigens. For years it was thought that the immune system was able to discriminate between self and non–self. However, with the growing knowledge of immunology, this theory has become more and more unsatisfactory. The self/non–self paradigm does not explain why perfectly healthy individuals can have circulating autoreactive T and B cells without any symptoms of autoimmune diseases.

Recently, a new concept providing more satisfactory explanations for the lack of autoimmune reactions in healthy individuals was developed. In this new hypothesis, the decision whether the immune system is activated does not solely depend on the recognition of an antigen as foreign, but also on the immune systems judgment whether it imposes danger to the integrity of the individual. The immune response must be considered as an outcome of a complex interaction between the lymphocyte and the antigen presenting cell (APC) in the context of cognate co–stimulatory signals and the local cytokine microenvironment in which the recognition of the specific antigen takes place. This new view on the immune system does not only provide explanations for issues that made us doubt about the self/non–self paradigm, it also provides more insight in the mechanisms of central and peripheral tolerance.

Th1 and Th2 cells: the role of Th1 cells in autoimmune diseases

Helper T cells regulate immune responses via cytokines that they produce upon

recognition of specific antigen presented by antigen presenting cells. Individual Th cells (clones) can be distinguished on the basis of the cytokine secretion profile and hence their function (Mosmann et al., *Annual Review of Immunology* 7: 145 (1989)). In response to most antigens, Th cells produce many cytokines simultaneously (type 0 cytokine profile). However, in response to certain types of antigens the Th cell response is biased to low levels of interferon-gamma (IFN-γ) and high levels of interleukin-4 (IL-4) and interleukin-5 (IL-5) (type 2 cytokine profile, Th2). In contrast, in response to certain other antigens, the production of cytokines of the Th cells is biased to high levels of IFN-γ and low levels of interleukin IL-4 and IL-5 (type 1 cytokine profile, Th1). There is accumulating evidence that type 1 and 2 profiles result from modulation of the local cytokine microenvironment (Trinchieri, *Immunology Today* 13: 379 (1993); Snijdewint et al., *J. Immunology* 150: 5321 (1993)). Various factors may directly act on the T cells, but they may also act indirectly by affecting antigen-presenting cells, which in turn secrete mediators that skew to Th1 or Th2 profiles.

Clearly, soluble factors secreted by antigen presenting cells during antigen-presentation are important. Antigen presenting cell-derived factors that skew T cell cytokine production towards Th1 and Th2 profiles include interleukin-12 (IL-12) and prostaglandin E2 (PGE-2). A low IL-12/PGE-2 production ratio in antigen presenting cells will results in IL-4 dominated T cell responses, whereas a high IL-12/PGE-2 production ratio will result in IFN-γ-dominated T-cell responses.

It is the current belief that many autoimmune diseases are caused by autoreactive Th1 cells. In experimental autoimmune models, the phenotype of T cells that induce disease has extensively been studied. Experimental autoimmune encephalomyelitis (EAE) is a model for multiple sclerosis. In this model that can be induced by transfer of T cells specific for central nervous system (CNS) antigens, the pathogenic T cells secrete a type 1 cytokine profile (Zamvil and Steinman, *Ann. Rev. Immunol.* 8: 579 (1990)). Likewise, in the non-obese diabetic (NOD) mouse model, transfer of T cells specific for a pancreatic autoantigen that had been differentiated in the presence of type 1 cytokines in vitro, caused disease, while the same T cells that had been differentiated in the presence of type 2 cytokines did not (Katz et al, *Science* 268: 1185 (1995)).

Evidence in humans for the mutual exclusive relationship between Th1 and Th2 response came from a recent study among Japanese school children that show a strong inverse relationship between delayed hypersensitivity responses to *M. tuberculosis* (Th1-type of response) and the presence of asthma, serum IgE levels and Th2-cytokine

profiles (Shirakawa et al., *Science* 275: 77 (1997)).

IL-12, a major regulator of type 1 T-cell cytokine responses

IL-12 is a heterodimeric glycoprotein composed of two covalently linked peptide chains, called p40 and p35 (Trinchieri *Ann. Rev. Immunol.* 13: 251 (1995)). IL-12 is mainly produced by activated monocytes and dendritic cells. IL12 can be produced by monocytes after stimulation with bacterial products such as LPS or after stimulation with activated T cells. For dendritic cells the ligation of CD40 with CD40L on the surface of activated T cells is the strongest trigger for IL-12 production. The most pronounced effect of IL-12 is the stimulation of IFN-γ by human NK cells and T cells. IL-12 exerts its effects through binding to a high affinity receptor. The functional, high-affinity IL-12 receptor (IL-12R) consist of a β1 and a β2 chain, of which only the latter is involved in signal transduction. The nucleotide and amino acid sequences of the IL-12 receptor β1 chain are disclosed in EP-A-638644. The sequences of the IL-12 receptor β2 chain are disclosed in EP-A-759466.

Immunotherapy for autoimmune diseases targeting autoreactive T cells

Currently used drugs for the treatment of autoimmune diseases are primarily directed at the treatment of symptoms. Most, if not all of these drugs are ineffective at stopping the disease process, need to be administered chronically and are often associated with significant side effects. This makes the presently used drugs highly unfavourable. Optimal drugs for the treatment of autoimmune diseases will be able to attenuate the autoimmune process by re-establishing the immune system's self-regulatory mechanisms that have failed and resulted in the autoimmune attack. Treatment during the early phase of the autoimmune process with such drugs have the potential to arrest the disease process.

It has been demonstrated that T cells play a central role in the auto-destructive process in autoimmune diseases such as rheumatoid arthritis (Sigall et al., *Clin. Exp. Rheum.* 6: 59 (1988)). Treatments that selectively suppress the activity of such autoreactive T cells can therefore be preferred. Such treatment could consist of the administration of an autoantigen or peptides derived thereof. This type of treatment has been very successful in the suppression of disease symptoms in various experimental autoimmune disease models in laboratory animals and it has been suggested that successful therapy is associated with the up-regulation of Th2 responses and a down-regulation of Th1 responses. It is therefore proposed by the present inventors to combine

antigen-specific therapy targeting autoreactive T cells with the modulation of the cytokine microenvironment.

Summary of the Invention

The current invention is based on the finding that Th2 cell development from naive Th cells is associated with suppression of IL-12R β2 chain expression leading to loss of IL-12 responsiveness and, consequently, the inability to promote IFN-γ production. Furthermore, the present invention is based on the finding that allergen-specific Th2 clones generated from atopic patients do not produce IFN-γ. Even upon exposure to IL-12, IFN-γ protein and mRNA expression cannot be induced in such clones. Further analyses revealed the complete lack of signalling via the IL-12R in these Th2 clones, as indicated by their inability to phosphorylate STAT4 despite the abundant presence of this selectively IL-12-induced transcription factor. FACS analysis showed normal expression of the IL-12R β1 chain. These findings strongly suggest the absence of functional β2 chains on human Th2 cells, similar to mouse Th2 cells. RNAse-protection assays with a human IL-12R β2 chain-specific DNA probe indeed indicated the absence of IL-12R β2 mRNA in activated Th2 clones.

Accordingly, the inventors propose to specifically neutralize the activity of the IL-12R β2 chain. Specific neutralization of the IL-12R β2 chain can be accomplished by a specific monoclonal antibody that binds to the IL-12R β2 chain, but does not stimulate the phosphorylation of STAT4. Such an antagonistic monoclonal antibody to the IL-12R β2 chain can be used to prevent or treat diseases in which activated type 1 T cells are involved. Such an antagonistic monoclonal antibody can be used to enhance the effect of antigen-specific therapy of autoimmune diseases targeting Th1-like autoreactive T cells.

Accordingly, it is a primary object of this invention to provide a pharmaceutical composition comprising a therapeutically effective amount of a monoclonal antibody capable of binding to the human IL-12R β2 chain, where said binding prevents interleukin-12 from mediating a signal through said receptor resulting in the phosphorylation of STAT4, in a pharmaceutically acceptable excipient.

It is an other objective of the present invention to combine a monoclonal antibody capable of binding to the human IL-12R β2 chain, where said binding prevents interleukin-12 from mediating a signal through said receptor resulting in the phosphorylation of STAT4, with specific autoantigens, modified autoantigens or peptide

fragments thereof.

It is an other objective of the present invention to combine a monoclonal antibody capable of binding to the human IL-12R β2 chain, where said binding prevents interleukin-12 from mediating a signal through said receptor resulting in the phosphorylation of STAT4, with other therapeutic monoclonal antibodies, such as monoclonal antibodies to co-stimulatory receptors on T cells or antigen presenting cells such as CD40, CD40L, CD80 and CD86.

It is a further objective of this invention to provide a method for treating autoimmune diseases, the method comprising administering to a patient in need of such treatment a therapeutically effective amount of a monoclonal antibody capable of binding to the human IL-12R β2 chain, where said binding prevents interleukin-12 from mediating a signal through said receptor resulting in the phosphorylation of STAT4, in a pharmaceutically acceptable excipient. In particular, said administration is combined with the administration of specific autoantigens, modified autoantigens or peptide fragments thereof, or, alternatively, with the administration of other therapeutic monoclonal antibodies, such as monoclonal antibodies to co-stimulatory receptors on T cells or antigen presenting cells, including CD4, CD40, CD40L, CD80 and CD86.

Detailed description of the invention

The invention pertains to antibodies, preferably monoclonal antibodies, capable of binding to the β2 chain of the IL12 receptor. The binding should be such that phosphorylation of a Signal Transducer and Activator of Transcription (STAT), specifically STAT4, is not activated. The activation of STATs by tyrosine phosphorylation in response to external stimuli such as cytokines was described by Schindler and Darnell, *Rev. Biochem.* 64: 621 (1995). Of the STAT molecules, STAT4 is the only one that is tyrosine phosphorylated after stimulation of T cells with interleukin 12. The molecular cloning of STAT4 based on its homology with STAT1 was described by Yamamoto et al, *Molec. Cell Biol.* 14: 4342 (1994). Antibodies that result in binding which prevents activation of STAT phosphorylation can be selected in a manner known per se, as exemplified in Example 5.

The invention further pertains to antibodies, preferably monoclonal antibodies, capable of binding to the β2 chain of the IL12 receptor, especially to an epitope of IL12R β2 chain, such that binding of the β2 chain to the IL12R β1 chain is prevented. Antibodies, the binding of which prevents (hetero)dimerization of the β1 chain to the β2

chain, can be selected e.g. by immunoprecipitation of the antibody–IL12R immuno–complex and comparison of the molecular weight of the immunocomplexes; antibodies resulting in immunocomplexes having the lower molecular weight complex (i.e. the complex which does not contain the heterodimer) are the ones sought according to the invention.

Particularly preferred are antibodies that prevent dimerization of β2 chain to β1 chain and also prevent activation of phosphorylation of STAT4.

As used herein, the term "antibody" refers to polyclonal antibodies, monoclonal antibodies, chimeric antibodies, humanized antibodies, single–chain antibodies, and fragments thereof such as Fab, F(ab')2, Fv, and other fragments which retain the antigen binding function of the parent antibody.

As used herein, the term "monoclonal antibody" refers to an antibody composition having a homogeneous antibody population. The term is not limited regarding the species or source of the antibody, nor is it intended to be limited by the manner in which it is made. The term encompasses whole immunoglobulins as well as fragments such as Fab, F(ab')2, Fv, and others which retain the antigen binding function of the antibody. Monoclonal antibodies of any mammalian species can be used in this invention.

As used herein, the term "chimeric antibodies" means that the constant regions of an immunoglobulin are derived from human immunoglobulin sequences.

As used herein, the term "humanized antibodies" means that at least a portion of the framework regions of an immunoglobulin are derived from human immunoglobulin sequences.

As used herein, the term "single chain antibodies or ScFv" refers to antibodies prepared by determining the binding domains (both heavy and light chains) of a binding antibody, and supplying a linking moiety which permits preservation of the binding function. This forms, in essence, a radically abbreviated antibody, having only that part of the variable domain necessary for binding to the antigen. Determination and construction of single chain antibodies are described in U.S. Patent 4,946,778, incorporated herein by reference.

As used herein, the terms "CD80", "CD86", "CD40" and "CD40L" refer to human surface molecules as extensively reviewed in Van Gool et al., *Immunol. Rev.* 153: 46 (1996), incorporated herein by reference. For immunization purposes CD80, CD86, CD40 and CD40L antigen may be prepared by any technique known in the art.

Antibodies to human CD80, CD86, CD40 and CD40L are known in the art. The present invention also contemplates a new use for such antibodies as detailed above.

As used herein, the term "autoantigen" refers to a human protein that is recognized by autologous T cells, resulting in self-tissue destruction in autoimmune disease patients. Examples of autoantigens that are recognized by autologous T cells are myelin basic protein in multiple sclerosis; collagen type II and human cartilage glycoprotein 39 (WO 96/13517) in rheumatoid arthritis; insulin and glutamic acid decarboxylase (diabetes); and alpha-fodrin (Sjögren's syndrome). For therapeutic use, autoantigens may be administered in there native form, modified by selected amino acid substitutions (WO 96/16085), or in peptide fragments with (Kumar et al., *Proc. Natl. Acad. Sci.* 87: 1337 (1990), both incorporated herein by reference) or without selected amino acid substitutions.

As used herein, the term "interleukin-12 receptor" refers to the human surface molecule capable of binding human interleukin-12 as reviewed above. For immunization purposes the human interleukin-10 antigen may be prepared by any technique known in the art.

As used herein, the term "antagonistic" refers to the capacity of a soluble ligand to bind to a cell surface receptor, where said binding prevents intracellular signal transduction leading to the activation of said cell surface receptor by the natural ligand for said.

The pharmaceutical compositions of this invention are administered at a concentration that is therapeutically effective to modulate the hosts immune response. To accomplish this goal, the pharmaceutical composition may be formulated using a variety of acceptable excipients known in the art. Typically, the pharmaceutical composition is administered by injection, either subcutaneous, intramuscular, intravenous or intra-peritoneal. Methods to accomplish this administration are known to those of ordinary skill in the art. It may also be possible to obtain compositions which may be orally administered, or which may be capable of transmission across mucous membranes. Before administration to patients, formulants may be added to the pharmaceutical composition.

The present invention will now be illustrated by reference to the following examples which set forth particularly advantageous embodiments. However, it should be noted that these embodiments are illustrative and are not to be construed as restricting the invention in any way.

EXAMPLES

Example 1

IL-12 modulates the production of IL-4, IL-5 and IFN-γ by stimulated CD4+ T cells

Naive CD45RA $CD4^+$ T cells were isolated from the heavy fraction of PBMC in a two step protocol. First $CD4^+$ cells were isolated by incubation with CD4 specific Dynabeads followed by Detatchabead treatment, as indicated by manufacturer (Dynal, Oslo, Norway). In the second step, UCHL-1 and HLA-DR positive cells were removed by panning, after labelling with appropriate antibodies. This procedure yielded a population of more than 98% $CD45RA^+$, $CD4^+$ T cells. These naive CD45RA $CD4^+$ T cells were stimulated in 96-well flat-bottom culture plates (Costar, Cambridge, MA) in Iscove's modified Dulbecco's medium (IMDM; Life Technologies, Paisley, UK), supplemented with 5 % pooled, C-inactivated normal human serum (CLB). To assess the direct modulatory effects of exogenous IL-12, T cells (2×10^4/well) were stimulated in the absence of accessory cells (AC) with a combination of immobilized anti-CD3 mAb (1 μg/ml), soluble anti-CD28 mAb (1 μg/ml) and with IL-2 (5 U/ml). IL-12 (200 U/ml) was added to the cultures at the start of the T cell stimulation. After 12 days of culture, resting T cells were harvested and restimulated with immobilized anti-CD3 mAb (1 μg/ml), soluble anti-CD28 mAb (1 μg/ml). Supernatants were harvested after 24 hours and analysed for the presence of cytokines by ELISA techniques as described by Van der Meide et al. (*J. Immunol. Methods* 79,293 (1985)) for IFN-γ, Van der Pauw-Kraan et al. (*Eur. Cytokine Network* 4: 343 (1993)) for IL-4 and McNamee et al. (*J. Immunol. Methods* 141: 81 (1991)) for IL-5.

In figure 1 it can be seen that addition of IL-12 during the priming of naive T cells strongly stimulates the production of the type 1 T-cell cytokine IFN-γ, but inhibits the production of the type 2 T-cell cytokines IL-4 and IL-5.

Example 2

Cloning of the human IL12R ß2 chain and expression on the surface of insect cells

The human IL12 receptor (IL12R) consist out of two chains, called ß1 and ß2. These form a heterodimer in order to act as a functional molecule on the cell-membrane. The ß2 chain is responsible for the transduction of signals into the IL12R expressing cells. The cDNA encoding the ß2 chain of the human IL12R was generated by PCR from RNA isolated from PBMC's. Briefly, the PBMC's were separated from red blood cells by gradient centrifuge using Ficoll, after which the lymphocyte fraction was stimulated

for 2 – 20 h with PMA (1ng/ml) and ionomycin (1μg/ml) in IMDM/FCS at 37°C with 5% CO2. Subsequently messenger RNA was prepared from the cells. The cells were washed twice with phosphate buffered saline (PBS pH 7.4) and lysed in 5M guanidinium thiocyanate in the presence of 0.7 M 2–mercaptoethanol. The RNA was bound on a Qiagen spin column, washed according to manufactures protocol and eluted in DEPC treated water. RNA was stored in –70°C.

First strand cDNA was synthesized by incubation at 37°C for 1 hour of 1–5 μg total RNA in a 50 μl mix, consisting of 1x synthesis buffer (USB), 0.5 mM dNTP, 5 μM random hexamers and 5 U M–MLV reverse transciptase (USB). This was followed by incubation at 70°C for 10 min. After cooling on ice from this mixture 2.5 μl was used as template in a PCR reaction using primers specific for respectively the IL12R ß2 chain. These primers (SEQ ID NO 1 and 2)were based on the published cDNA coding sequences for IL12R ß2 (Presky D.H. et al., *Proc. Natl. Acad. Sci. USA* 93: 14002 (1996)).

SEQ ID NO 1 Sense primer: 5' – gcgcgaattc ttgttgatgg cacatacttt tag – 3'

SEQ ID NO 2 Antisense primer: 5' – gcgccccggg tcagagcatg agggagtcac acc – 3'

Both the sense and the anti–sense primers start with GCGC followed by a restriction site for cloning purpose. The sense primers carriers the ATG start codon, while the anti–sense primers contains a stop codon. The amplified cDNA will encode for the full–length IL12R ß2 chain including the naturally occurring signal peptide. To amplify the IL12R ß2 chain a standard PCR was done. The PCR mixture of 100 μl contained 1x PCR buffer, 2.5U Taq polymerase, 0.25 mM dNTPs, 25 pmole of each primer and 2.5 μl cDNA template. The mixture was run in Perkin Elmer thermocycler for 20 – 40 cycles of 1 min 95°C, 1 min 55°C, and 2 min 72° C followed by 1 step for 7 min at 72°C as extension of the PCR product. The obtained PCR product was gel purified and cloned in pCR Script using the Stratagene cloning kit. Briefly, the PCR product was incubated with plasmid together with T4 ligase and SrfI for 1h at KT, after which the entire sample was transformed in Xl1Blue E.coli cells. The cells were plated on LB plates containing 100 μg ampicillin/ml, 20 μg IPTG/ ml and 20 μg Xgal/ml. After incubation over night at 37°C putative white clones were analysed for having an insert. Clones containing inserts were analysed by cycle sequencing using M13 and M13 reverse primers. Several clones were identified containing a DNA sequence encoding for the IL12R ß2 chain. By further sequencing a correct cDNA clone encoding full–length Il12R ß2 chain was found without PCR induced mutations.

To express the IL12R ß2 chain on the cell surface of Sf9 insect cells, the obtained cDNA was re-cloned in the baculovirus transfer vector pVL1392. The pVL1392 vector and the IL12R ß2 chain cDNA cloned in pCR Script were digested with EcoRI and SmaI. The IL12R ß2 chain insert and the linear pVL1392 were gel purified, after which the insert was ligated in pVL1392. The ligation mixture contained 100 ng plasmid, 100 ng insert, 1x ligase buffer and T4 DNA ligase (Promega). The ligation mixture was transformed to DH5a, and plated on LB plates containing 100 μg ampicillin/ml. After incubation over night at 37°C the clones were screened for having the correct plasmid. A pVL1392 plasmid containing the IL12R ß2 chain insert was selected and large scale plasmid preparation was done using the midi-prep system from Qiagen.

Example 3

Baculovirus expression of human IL12R β2 chain and generation of monoclonal antibodies.

Using the transfer vector pVL1392 containing the full-length IL12R ß2 chain cDNA, obtained in example 2, the sequence was recombined into the *Autographa californica* baculovirus (AcNPV). Briefly, using the BaculoGold system from Pharmingen the recombinant plasmid was cotransfected at a 4 to 1 ratio with wild-type baculoviral DNA containing a lethal deletion into Sf9 (Spodoptera frugiperda) insect cells. Recombinant baculovirus was plaque purified, followed by several rounds of amplification to obtain a high titer recombinant virus stock.

For the generation of monoclonal antibodies Sf9 cells were infected with the IL12R ß2 chain carrying virus. Sf9 cells were infected with recombinant virus at a MOI of 10. The cells were harvested after 48-72 hours of culture in TC100 FCS at 28°C under standard conditions. The cells were washed with PBS twice followed by injection intraperitoneally in female BALB/c mice (5 x 10^6 Sf9 IL12R $\beta2^+$ cells/ mouse). At day 14, 21, 28 and 100 the mice received a new booster injection with Sf9 IL12R ß2 $^+$ cells. Three days after the last injection the spleen cells from one mouse were isolated and used for cell fusion at a ratio of 10 : 1 with Sp2/0 murine myeloma cells using 38% polyethylene glycol. The fused cells were resuspended in IMDM/FCS supplemented with HAT, followed by plating on ten 96 wells plates. After 10 – 14 days the fusion was screened for grow and antibody production. Therefore the supernatants of each row (1-12) and separately the supernatants of each column (A-H) of a 96 well plate were pooled, resulting in 200 samples. These samples were screened in a FACS analysis using

zero in 100 µl FACS buffer (PBS pH 7.4 1% BSA 0.1% NaN_3).

After washing to remove non bound antibody the cells were incubated for another 20 min at 4°C with the in example 3 described FITC antibody. The cells were washed with FACS buffer and finally suspended in FACS buffer containing 0.5% para-formaldehyde and analyzed with a FACScan flow cytometer (Becton Dickinson). A dose dependent correlation from Mab 3H4 with the MFI was observed, indicating that 3H4 specifically binds to the Hut78.8 cells (figure 2). To further assess the specificity of 3H4, besides Hut78.8 also JY and Jurkat cells were stained with the 3H4 Mab (figure 3). Finally, the influence of PMA/ionomycin and PHA in combination with IL12 and anti-IL4, on the upregulation of the IL12R β2 on naïve T-cells was investigated. Briefly, $CD45RA^+$ $CD4^+$ T-cells were isolated as described in example 1, followed by an incubation for 3 days with the stimuli as indicated in the legend of figure 4. Subsequently the cells were washed and analyzed by FACS using the 3H4 Mab as described above. In the same experiment freshly isolated $CD45RA^+$ $CD4^+$ T-cells were used as control cells. As clearly is shown in figure 4, stimulation of the T cells upregulate the IL12R β2 chain which can be recognized by Mab 3H4.

Example 5

Characterization of anti-IL-12R β2 monoclonal antibodies

For analysis of IL-12-induced tyrosine phosphorylation of STAT4, $5x10^6$ TLC cells are or are not exposed to IL-12 (100 U/ml) for 20 min. in the absence or presence of anti-IL-12R monoclonal antibodies, washed twice with ice-cold PBS and lysed in 250 µl of immunoprecipitation buffer (IPB) [10 mM Tris/HCl (pH 7.4), 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1 % Nonidet P-40, 0.25 % sodium deoxycholate, 10 µg/ml aprotinin, 10 µg/ml leupeptin, 1 mM PMSF, 1mM sodium orthovanadate and 10 mM NaF]. Lysates are precleared by three incubations with 50 µl of a 10% (v/v) suspension of non-immune mouse Ig-coated protein A-CL4B Sepharose beads (Pharmacia, Uppsala, SE), and once with uncoated protein A-Sepharose beads. Precleared lysates are then incubated with anti-STAT4 (C20, Santa Cruz) for 30 min followed by protein A-Sepharose beads for 2 h. After washing in IPB, the STAT4 immunoprecipitates are resuspended in sample buffer, separated by SDS-PAGE under reducing condition, and transferred to Hybond C nitrocellulose membrane (Amersham Co., Aylesbury, UK), employing a semidry electroblotting chamber (Multiphore II, Pharmacia, SE). Blots are saturated with blocking buffer [50 mM Tris, 150 mM NaCl (pH 7.5) containing 0.2 %

PMA/ionomycin stimulated $CD4^+$ T cells resulting in 57 positive samples. Briefly, T cells (0.1 - 0.2 x 10E6/sample) were incubated for 20 min. at 4°C with the pooled supernatants. After washing with FACS buffer (PBS pH 7.4 1% BSA 0.1% NaN_3), the cells were incubated for another 20 min at 4°C with goat anti-mouse antibodies conjugated to fluorescein isothiocyanate (FITC). The cells were washed with FACS buffer and finally suspended in FACS buffer containing 0.5% paraformaldehyde and analyzed with a FACScan flow cytometer (Becton Dickinson). Subsequently, the supernatants of positive pools were individually tested in the same assay using stimulated $CD4^+$ T cells and using a T-cell clone L70 which consistently express the IL12R ß2 chain. Furthermore the isotype of each selected hybridoma was determined. As final result 1 hybridoma (clone 3H4) was obtained of the IgG1 isotype against the IL12R ß2 chain. Results of the fusion are summarized in table 1.

Fusion	wells	wells with grow	positive wells first screen	positive wells second screen	IgM	IgG1 κ
	960	760	57	15	14	1

Table 1. Lymphocytes were fused with Sp2/0 cells, plated on ten 96 wells plates and screened for grow. After FACS analysis 14 IgM and 1 IgG producing hybridoma clones were selected.

Hybridoma clone 3H4 was subcloned three times by limiting dilution in IMDM/FCS + IL6 (100U/ml). The selected positive hybridoma 3H4 was scaled up for production of the antibody. The obtained monoclonal antibody was initially characterize by doing FACS analysis on various type of cells (B- and T cells; dendritic cells; monocytes).

Example 4

Characterization of anti-IL12R β2 monoclonal antibody 3H4.

First the purified monoclonal antibody was titrated in FACS analysis on a non stimulated T-cell line Hut78.8. This cell line was selected based on the constitutively presence of mRNA coding for the IL12R β2 chain. The Hut78.8 cells (100,000 cells) were incubated for 20 min. at 4°C with Mab 3H4 ranging from 400 ng/100,000 cells to

Tween and 1% BSA] and incubated with horseradish peroxidase-labelled anti-phosphotyrosine (RC20; Signal Transduction Laboratories, Lexington, KY) for 1 h. Phosphorylated tyrosine residues are visualized using enhanced chemiluminescence (ECL, Amersham). For detection of STAT4 proteins on the same blots, deprobing of the blots is performed according to the manufacturer's instructions. Blots are then incubated with anti-STAT4 (C20, Santa Cruz Biotechnology) for 1 h, washed, incubated for 1 h with horseradish peroxidase-labelled horse anti-rabbit Ig (CLB), and visualized as described above. It is demonstrated that the specific anti-IL12R ß2 monoclonal antibodies can prevent the phosphorylation of STAT4 and thus are potent inhibitors of the signal transduction cascade in lymphocytes leading to a strong type-1 pro-inflammatory cytokine production.

Description of the figures

Figure 1 shows the effect of addition of IL-12 during the priming of naive T cells. IL-12 stimulates the production of the type 1 T-cell cytokine IFN-γ, but inhibits the production of the type 2 T-cell cytokines IL-4 and IL-5.

Figure 2 shows an FACS analysis of Mab 3H4 on Hut78.8 cells. 100,000 cells were incubated with Mab 3H4 in titration followed by detection with an anti-mouse FITC-labelled antibody.

Figure 3 shows an FACS analysis of Mab 3H4 on Hut78.8, JY and Jurkat cells. 100,000 cells were incubated with Mab 3H4 (500 ng) followed by detection with an anti-mouse FITC-labelled antibody. In each figure, the overlay is the control incubation in which only the secondary antibody was used in the FACS analysis.

Figure 4 shows an FACS analysis of Mab 3H4 on naive T cells (control), naive T cells after stimulation for 3 days with PMA (1 ng/ml) and ionomycin (1 µg/ml) and on naive T cells after stimulation for 3 days with PHA (1 ng/ml), IL12 and anti-IL4. After culture, 100,000 T cells were incubated with Mab 3H4 (500 ng) followed by detection with an anti-mouse FITC-labelled antibody. In each figure, the overlay is the control incubation in which only the secondary antibody was used in the FACS analysis.

Claims

1. A monoclonal antibody that can bind to the IL12R ß2 chain expressed on the cell surface of human T lymphocytes, where said binding prevents IL12R ß2 chain–mediated STAT4 phosphorylation.

2. A monoclonal antibody that can bind to the IL12R ß2 chain expressed on the cell surface of human T lymphocytes, where said binding prevents the IL12R ß2 chain from dimerization to the IL12R ß1 chain.

3. A monoclonal antibody that can bind to the IL12R ß2 chain expressed on the cell surface of human T lymphocytes, where said binding prevents IL12R ß2 chain–mediated STAT4 phosphorylation and prevents the IL12R ß2 chain from dimerization to the IL12R ß1 chain.

4. A combination of a monoclonal antibody of any one of claims 1–3 or part thereof, and an autoantigen, peptide fragments of an autoantigen or a modified form thereof.

5. A combination according to claim 4, wherein said autoantigen is selected from myelin basic protein, collagen type II, human cartilage glycoprotein 39, heat shock proteins, insulin, glutamate decarboxylase and α–fodrin.

6. A combination of a monoclonal antibody of any one of claims 1 or part thereof and a second monoclonal antibody.

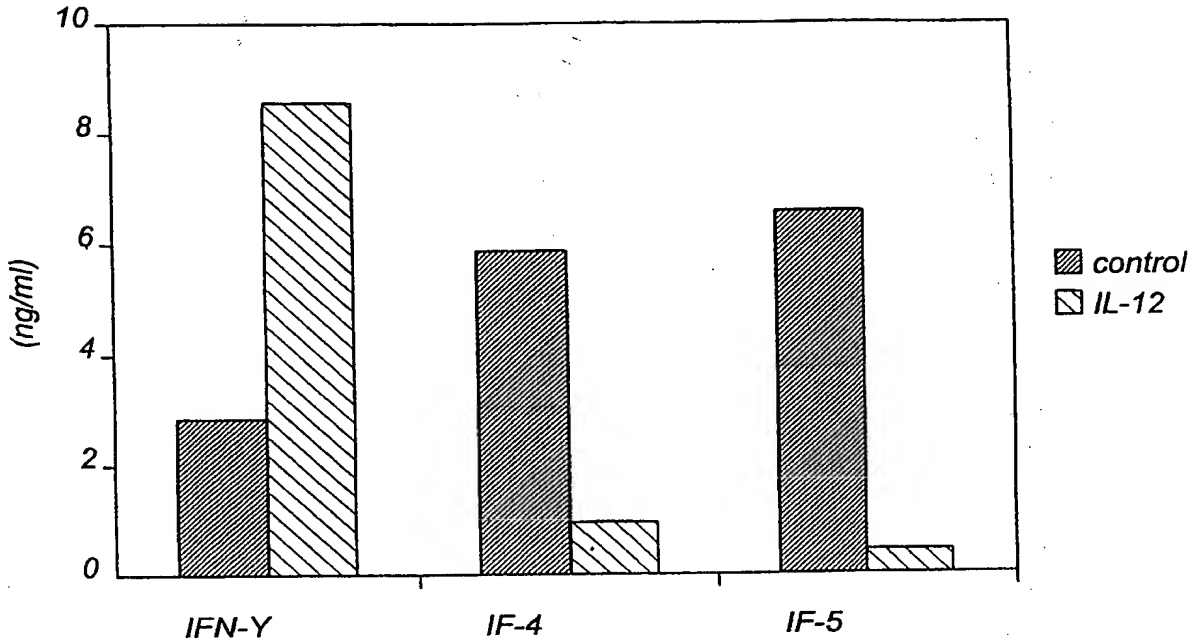
7. A combination according to claim 6, wherein said second monoclonal antibody is selected from antibodies to co–stimulatory receptors on T cells or antigen presenting cells, especially CD40, CD40L, CD80 and CD86.

8. A pharmaceutical composition comprising the antibody of any one of claims 1–3 or the combination of any one of claims 4–7.

9. A pharmaceutical composition according to claim 8, comprising a heat shock protein or peptide fragments of said heat shock protein for the stimulation of type 2 cytokine producing regulatory T cells.

10. A method for treating autoimmune diseases, the method comprising administering to a patient in need of such treatment a therapeutically effective amount of a pharmaceutical composition of claim 8 or 9.

Fig 1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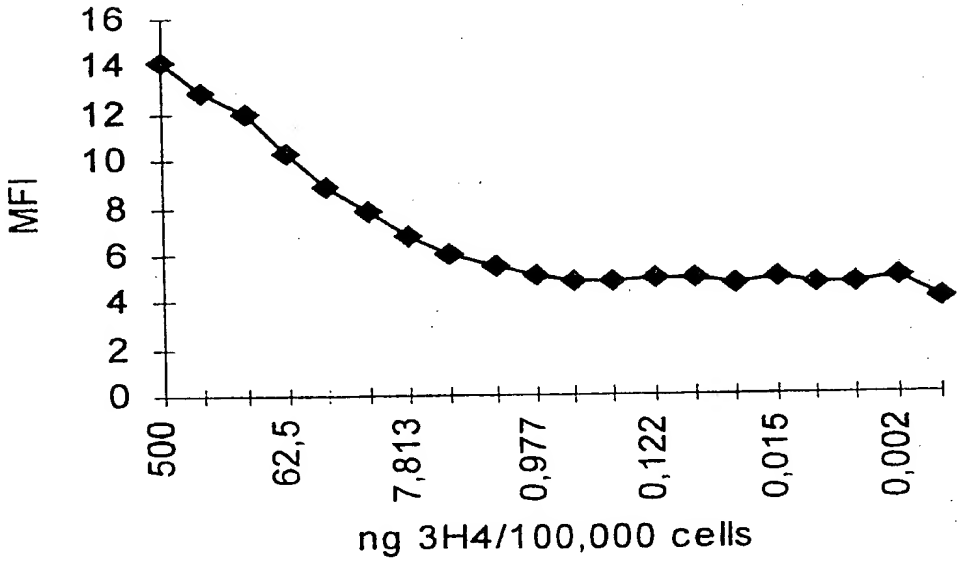
(ng/ml)
control
IL-12
IFN-Y
IF-4
IF-5

Fig 2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10
8
6
4
2
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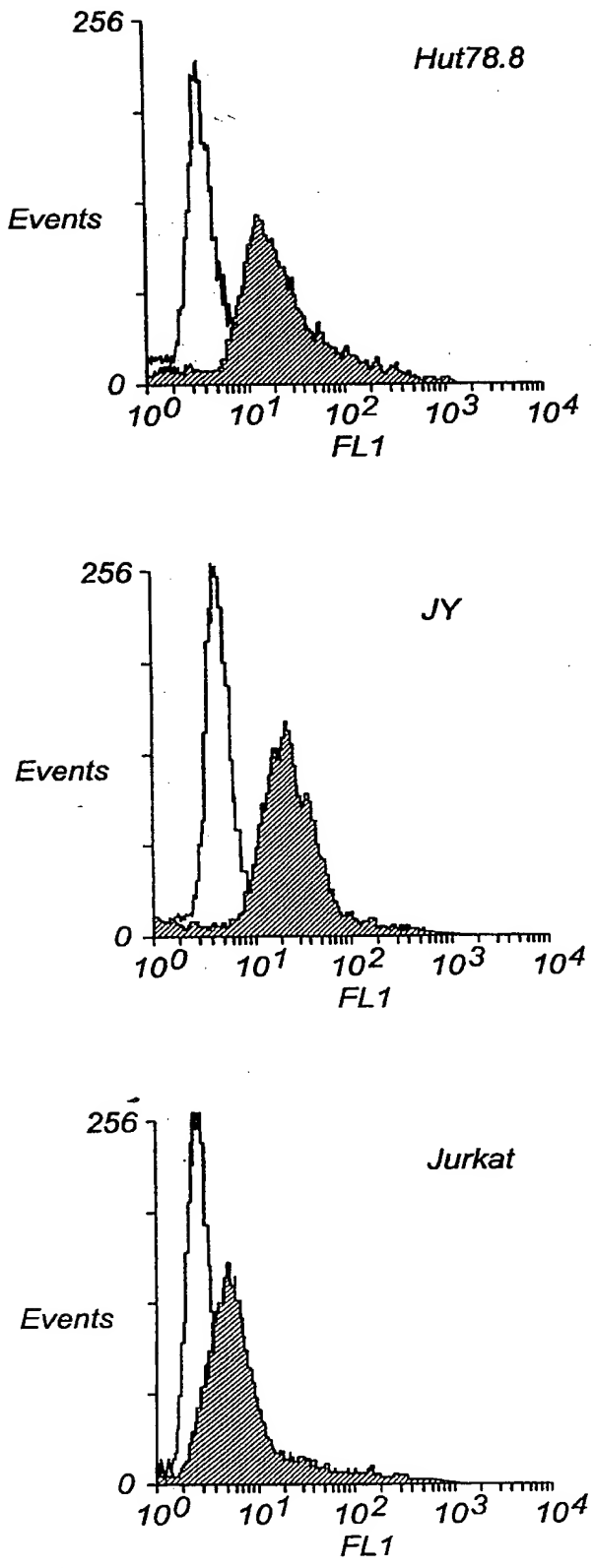
0,002
ng 3H4/100,000 cells

Fig 3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256
JY
Events
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FL1
256
Jurkat
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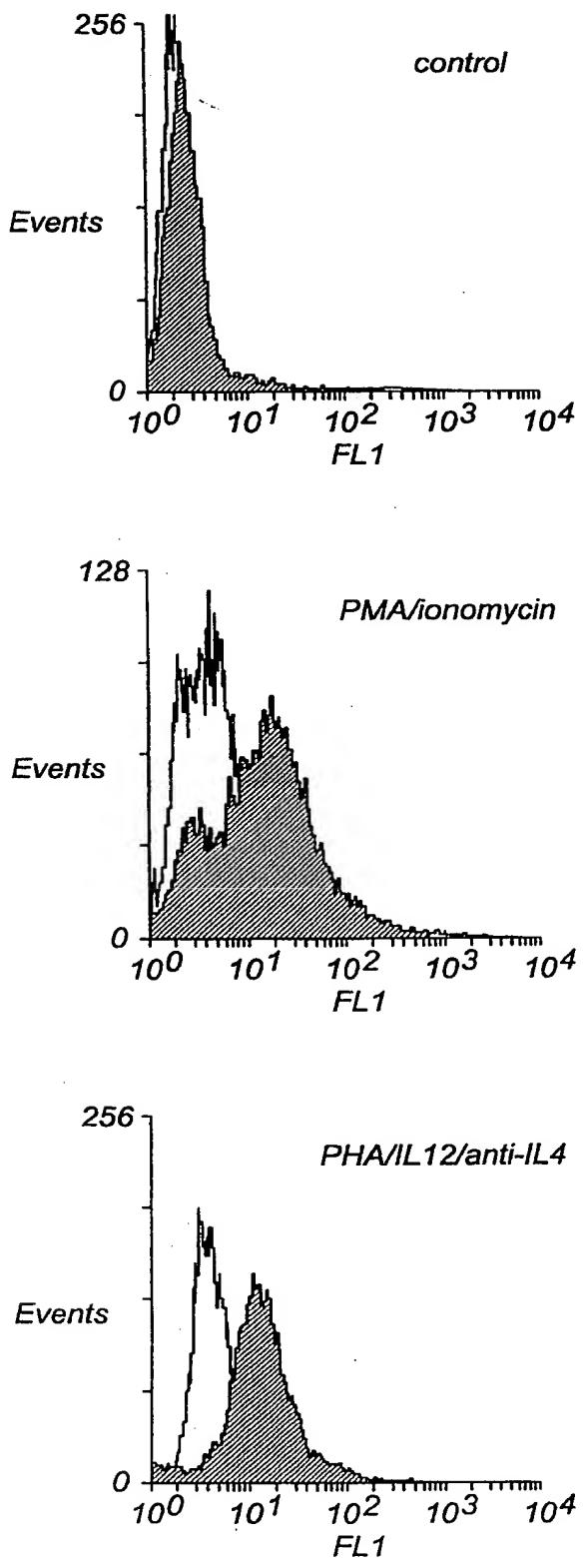
FL1

Fig 4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PHA/IL12/anti-IL4
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INTERNATIONAL SEARCH REPORT

information on patent family members

Inter onal Application No
PCT/NL 98/00663

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0759466 A	26-02-1997	JP 9132598 A	20-05-1997
US 5852176 A	22-12-1998	US 5840530 A	24-11-1998
US 5853721 A	29-12-1998	NONE	
WO 9841232 A	24-09-1998	AU 6760498 A	12-10-1998
EP 0638644 A	15-02-1995	US 5536657 A	16-07-1996
		AU 676325 B	06-03-1997
		AU 6750594 A	27-01-1995
		CA 2128151 A	20-01-1995
		JP 7194383 A	01-08-1995
		NZ 264003 A	21-12-1995
		ZA 9405154 A	19-01-1996
		US 5831007 A	03-11-1998
US 5639858 A	17-06-1997	AU 5368396 A	08-10-1996
		CA 2215745 A	26-09-1996
		EP 0815133 A	07-01-1998
		WO 9629341 A	26-09-1996
		US 5756700 A	26-05-1998